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## JASN

**IgA1 GLYCOSYLATION IS HERITABLE IN HEALTHY TWINS**

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IgA1 Glycosylation Is Heritable in Healthy Twins

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**ABSTRACT**

IgA nephropathy (IgAN) is the most common form of primary GN and an important cause of kidney failure. Characteristically, patients with IgAN have increased serum levels of undergalactosylated IgA1 (gd-IgA1). To assess the degree to which serum gd-IgA1 levels are genetically determined in healthy individuals, we determined serum IgA and gd-IgA1 levels by ELISA in a sample of 148 healthy female twins, including 27 monozygotic and 47 dizygotic pairs. Using the classical twin model, we found the heritability of serum gd-IgA1 and IgA levels to be 80% (95% confidence interval: 66% to 89%) and 46% (95% confidence interval: 15% to 69%), respectively. These data indicate that serum gd-IgA1 levels are highly heritable. Elucidating the genetic basis of this heritability will be important in understanding the pathogenesis of IgAN.

MAIN TEXT

IgA Nephropathy (IgAN) is the most frequently diagnosed type of glomerulonephritis in the world.<sup>1</sup> The course of disease is complex and not yet fully understood. Prognosis is variable. Some patients have a very mild form of the disease that requires little to no treatment. However, others have progressive disease with up to 50% of patients developing end stage renal failure within 20 years of diagnosis. IgAN recurs in approximately 50-60% of transplanted patients, indicating an important contribution of extra-renal factors to pathogenesis.<sup>2,3,4,5</sup> A great deal of evidence exists to support a significant genetic contribution to IgAN.<sup>5,6,7,8,9</sup> The incidence of IgAN varies geographically, being most prevalent in East Asian populations and less prevalent in European and African populations.<sup>10,11</sup> Six genome wide association studies (GWAS) have collectively identified 20 distinct loci associated with IgAN. Interestingly, most of these loci are shared with other immune-related diseases.<sup>12,13,14,15,16,17</sup> One associated SNP has been located within the *ST6GAL1* gene.<sup>16</sup> *ST6GAL1* encodes ST6 beta-galactosamide alpha-2,6-sialyltransferase 1, a glycosyltransferase. However, none of the loci are specifically associated with genes involved in IgA1 glycosylation. One of the hallmarks of IgAN is the presence of increased amounts of circulating under-galactosylated IgA1 antibodies. Usually, glycans on the hinge region of IgA1 terminate with galactose. In IgAN patients an increased proportion of IgA1 glycans terminate in *N*-acetylgalactosamine (GalNAc) or sialylated GalNAc.<sup>5,18</sup> This type of IgA1 is termed 'galactose-deficient' IgA1 (gd-IgA1). Galactose-deficient IgA1 has an established role in the development of IgAN. In the proposed 4-hit hypothesis of IgAN pathogenesis, an increase in gd-IgA1 triggers the production of anti-glycan auto-antibodies.<sup>8,19,20</sup> This leads to the formation of immune complexes that, under this hypothesis, may deposit in the kidney and cause kidney injury.<sup>21,22</sup> Levels of gd-IgA1 are elevated in IgAN patients, regardless of ethnicity or age.<sup>23,24,45</sup>

Studies of familial IgAN have provided heritability estimates for gd-IgA1 between 54% and 76%.<sup>25,26,27</sup> One difficulty of these studies is that at-risk relatives tend to show increased gd-IgA1 levels, biasing the heritability estimates, which have been suggested to strongly depend on the gd-IgA1 levels of the index IgAN case.<sup>26</sup> In order to understand the genetic contribution to gd-IgA1 levels in IgAN patients, it is first necessary to understand the genetic contribution to gd-IgA1 levels in healthy individuals. The classical twin model

allows the estimation of the environmental and genetic contribution to phenotypic variation. We assessed the heritability of serum gd-IgA1 and IgA levels in a randomly ascertained sample of 148 healthy female twins from the TwinsUK cohort consisting of 47 dizygotic and 27 monozygotic pairs. All individuals were female of white Caucasian ethnicity. The mean age was 56.9 years (range=27.1-84.8, SD=13). Phenotypic characteristics are summarised in Table 1.

We measured IgA and gd-IgA1 levels in serum by ELISA. To demonstrate consistency among multiple measurements of both IgA and gd-IgA1, we designed a fully-crossed experiment, where 58 randomly selected control samples were assessed on three testing days. We assessed the intra-rater reliability of each experiment by calculating the intra-class correlation coefficient (ICC).<sup>28</sup> The ICC for the IgA assay was 0.74 (95% CI: 0.63-0.83) and the ICC for the gd-IgA1 assay was 0.89 (95% CI: 0.73-0.95), showing a good to excellent reproducibility.<sup>29</sup> We next assessed serum IgA and gd-IgA1 levels in our Twin cohort. The mean serum IgA level was 3.16 mg/ml (range 0.91 to 5.41, SD=0.73). The mean serum gd-IgA1 level was 0.54 AU (range 0.21 to 0.89, SD=0.15). For both parameters the data were normally distributed ( $P>0.05$ , Shapiro-Wilk normality test). To control for potential batch effects analyses were carried out using the plate-adjusted residuals for both traits and age at assessment was included in all models. To determine longitudinal stability of gd-IgA1, we analysed gd-IgA1 levels in two samples from each individual ( $n=40$  individuals). The samples were collected 5 years apart. There was no difference in HAA binding of the paired samples over time (Figure 1,  $r=0.92$ ,  $p<0.0001$ ). This data demonstrated longitudinal stability of gd-IgA1 in the twins and is consistent with previous studies (reviewed in <sup>30</sup>).

We fitted three different nested genetic models to the data using OpenMX: a) the E model, which assumes that the phenotypic variability in the population is determined only by the Environment; b) the AE model which assumes that both Additive genetic effects and the Environment play a role; c) the ACE model that includes an additional component for the Common shared familial environment. The AE model was the best-fitting model for the estimation of both gd-IgA1 and IgA level heritability ( $AIC_{ACE}=-485.6$ ;  $AIC_{AE}=-487.5$  and

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2  
3 AIC<sub>ACE</sub>=11.0; AIC<sub>AE</sub>=9.0, respectively). Additive genetic effects accounted for 80.4% (95% CI: 65.6-88.7%) of  
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5 the variance of gd-IgA1 levels and individual-specific environment effects explained the remaining 19.6%  
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7 (95% CI: 11.3-34.4%) of the variance. Additive genetic effects accounted for 46.3% (95% CI: 15.2-68.6%) of  
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9 the variance of IgA levels, and individual-specific environment effects explained the remaining 53.7% (95%  
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11 CI: 31.4-84.8%) of the variance. These data show that, unlike serum IgA, serum gd-IgA1 is highly heritable.  
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13 This is confirmed by the fact that, in contrast to serum IgA levels, the correlation of gd-IgA1 between  
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15 monozygotic twins ( $r=0.84$ ) was much higher than the correlation between dizygous twins ( $r=0.46$ ; Figure 2).  
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17 Our analyses suggest that the variability of gd-IgA1 levels in the general healthy population is strongly  
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19 determined by genes with additive effect on the trait, while the individual environment (lifestyle, exposure)  
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21 plays a much smaller role. Analogously, no effects due to common environmental or lifestyle factors that are  
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23 shared within each family were identified in our sample. Overall, our data show that circulating gd-IgA1 is  
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25 highly heritable (80.4%) in a healthy population, indicating that serum gd-IgA1 levels are under strong  
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27 genetic control. Heritability of gd-IgA1 has previously been demonstrated in studies based on families  
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29 ascertained through the presence of IgA nephropathy or Henoch-Schonlein Purpura, though estimates were  
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31 sometimes dependent on the gd-IgA1 levels of the index case.<sup>25,26,27</sup> An increased level of gd-IgA1 is  
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33 associated with IgAN and is considered to be the ‘first hit’ in the proposed disease pathogenesis model.<sup>8,20</sup>  
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35 Notably, asymptomatic first-degree relatives of IgAN patients have high gd-IgA1 levels, suggesting that  
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37 additional factors (‘hits’) are required for IgAN to develop. Consistent with previous studies, serum IgA levels  
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39 showed low heritability (46.3%) in our cohort.<sup>31,32</sup> In conclusion, our study found gd-IgA1 levels to be a  
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41 highly heritable trait in the general population, with a heritability estimate of about 80%. Disentangling the  
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43 genetic component underlying gd-IgA1 variability may help the identification of genetic risk factors for IgAN  
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45 susceptibility.  
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## CONCISE METHODS

### *Sample Cohort*

The TwinsUK adult twin registry includes about 12,000 subjects, predominately white Caucasian females unselected for any specific disease, and recruited from all over the UK from 1992. Individuals from the TwinsUK cohort have been shown to have similar disease and lifestyle characteristics to the general population.<sup>33</sup> St. Thomas' Hospital Research Ethics Committee approved the study, and all twins provided informed written consent. The sample used for this study was randomly ascertained among healthy twin pairs. All the data is available upon request from the Twin Research Unit website ([www.twinsuk.ac.uk/data-access/submission-procedure/](http://www.twinsuk.ac.uk/data-access/submission-procedure/)).

### *Measurement of Serum IgA*

Serum IgA levels were measured by ELISA.<sup>23</sup> MaxiSorb immunoplates (Nunc; Life Technologies) were coated overnight at 4°C with 3 µg/ml F(ab')<sub>2</sub> fragment goat anti-human IgA (Jackson) in coating buffer (0.05 M Na<sub>2</sub>CO<sub>3</sub> pH 9.6). In between each incubation step, plates were washed 3 times with washing buffer (PBST; 0.1% Tween 20). Plates were blocked for 1 hour at room temperature with carbofree (Vector Labs). Samples were diluted 1/80,000 in carbofree and incubated at room temperature for 2 hours. Detection was carried out for 1 hour at room temperature using F(ab')<sub>2</sub> fragment biotinylated goat anti-human IgA1 (Jackson Immunoresearch), followed by Extravidin-HRP. ELISAs were developed using TMB substrate (BD Biosciences) and absorbance was measured at 450 nm. A standard curve was produced on each plate using serial dilutions of purified IgA1 (Abcam) from 100 ng/ml – 1.56 ng/ml.

### *Measurement of gd-IgA1*

Levels of serum gd-IgA1 were measured using a lectin-based ELISA.<sup>34</sup> MaxiSorb immunoplates were coated overnight at 4°C with polyclonal rabbit anti-human IgA (Dako) diluted 1/1000 in coating buffer (0.05 M Na<sub>2</sub>CO<sub>3</sub> pH 9.6). In between each incubation step, plates were washed 4 times with washing buffer (PBST; 0.1% Tween 20, 0.355 M NaCl). Plates were blocked for 1 hour at room temperature with carbofree (Vector



Labs) and samples were diluted 1/100 in PBS (to ensure saturation of IgA) and incubated overnight at 4°C. HAA-biotin (Sigma) diluted 1/1000 in PBS was added to each well for 90 min at room temperature, followed by poly-streptavidin HRP (Pierce) diluted 1/10,000 also for 90 min at room temperature. ELISAs were developed using TMB substrate (BD Biosciences) and absorbance was measured at 450 nm. Three control samples were run on each plate (“low”, “medium” and “high”), to test for inter- and intra- assay variation.

**Heritability Estimation**

We used openMX (<http://openmx.psyc.virginia.edu/>; version 2.2.4) to estimate the contribution of additive genetic (A), shared (C) and individual-specific environment (E) effects on serum IgA and gd-IgA1 level variation (ACE model).<sup>35</sup> We also compared the ACE model with the most parsimonious AE model, which does not include the effect of common environment influences, assuming that all familial aggregation results from additive genetic effects, and against the E model that assumes all variability to be determined by the environment. The models were compared through Akaike's information criterion (AIC) in order to determine which model attained the best goodness-of-fit in the most parsimonious way. In the analyses the age at serum level collection was included as covariate.

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**DISCLOSURES**

None

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FIGURE LEGENDS

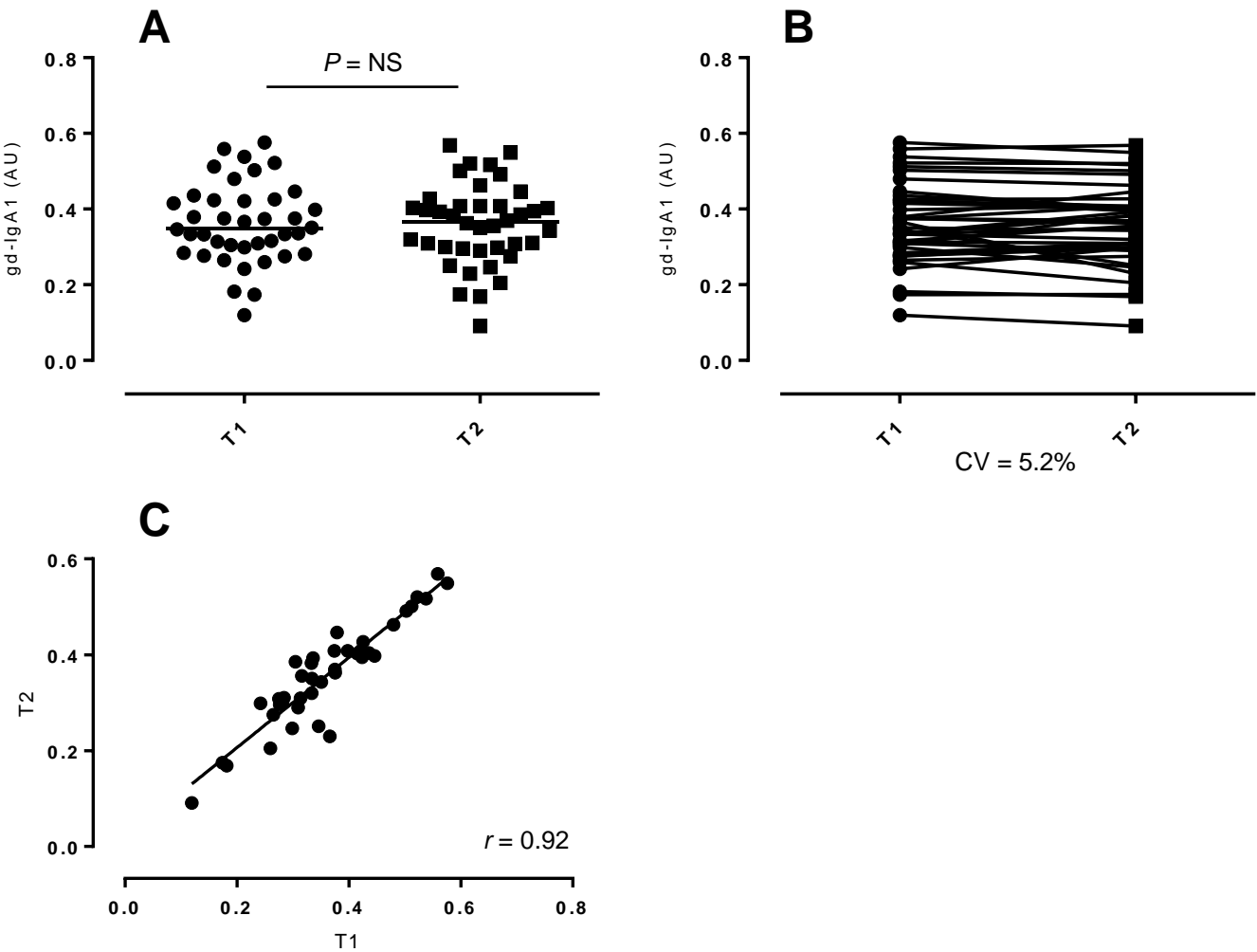
**Figure 1. Stability of gd-IgA1 levels over time.** Unpaired (A, bars represent group median) and paired (B) scatter plots show gd-IgA1 levels determined at 2 time points, T1 and T2 (5 years apart), for individual samples (n=40 individuals with paired samples from each). There was no difference in gd-IgA1 levels in the paired samples over time (paired t-test,  $P=NS$ ), with a good correlation between T1 and T2 (C,  $r=0.92$ ,  $P<0.0001$ , Pearson's correlation).

**Figure 2. Twin by twin scatterplots.** Serum gd-IgA1 levels in monozygotic (MZ; A) and dizygotic (DZ; B) twins and of serum IgA levels in MZ (C) and DZ (D) twins. The levels of gd-IgA1 were more correlated in MZ twins than in DZ twins; Intrapair correlations were 0.84 ( $P < 0.0001$ ) and 0.46 ( $P=0.001$ ) respectively. These data suggest gd-IgA1 level is highly heritable. Conversely, intrapair correlations of IgA level were 0.48 ( $P = 0.01$ ) and 0.30 ( $P=0.041$ ) indicative of IgA level being less heritable than gd-IgA1 level.

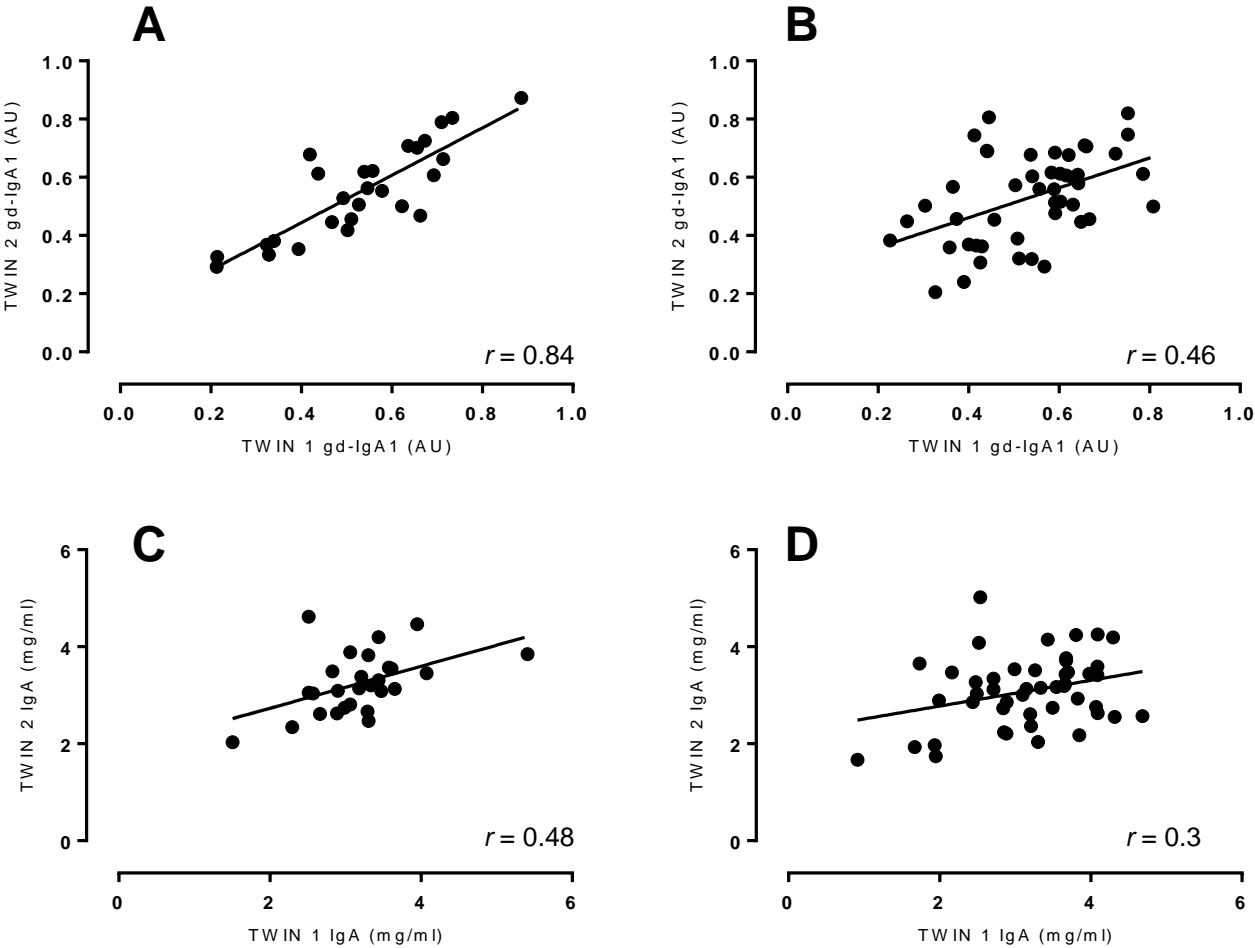
**Table 1.** Phenotypic details of the 148 female Caucasian individuals in the study sample.

		Mean	Standard deviation	1 <sup>st</sup> quantile	3 <sup>rd</sup> quantile
<b>Monozygotic</b>	N=54				
<b>Age</b>	(years)	52.73	12.16	45.14	62.86
<b>IgA</b>	(mg/ml)	3.22	0.65	2.81	3.54
<b>gd-IgA1</b>	(AU)	0.54	0.16	0.42	0.66
<b>Dizygotic</b>	N=94				
<b>Age</b>	(years)	59.28	12.98	48.91	69.76
<b>IgA</b>	(mg/ml)	3.13	0.77	2.62	3.67
<b>gd-IgA1</b>	(AU)	0.53	0.15	0.43	0.63

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Lomax-Browne *et al.* Figure 1.



Lomax-Browne *et al.* Figure 2.